Faecal particle size: digestive physiology meets herbivore diversity

Clauss, Marcus ; Steuer, Patrick ; Erlinghagen-Lückerath, Kerstin ; Kaandorp, Jacques ; Fritz, Julia ; Südekum, Karl-Heinz ; Hummel, Jürgen

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3 Marcus Clauss[a], Patrick Steuer[b], Kerstin Erlinghagen-Lückerath[b], Jacques Kaandorp[c], Julia
4 Fritz[a], Karl-Heinz Südekum[b,*], Jürgen Hummel[b,d]
5
6 [a]Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich,
7 Switzerland
8 [b]Institute of Animal Science, University of Bonn, Endenicher Allee 15, 53115 Bonn,
9 Germany
10 [c]Safari Park Beekse Bergen, Hilvarenbeek, The Netherlands
11 [d]Department of Animal Sciences, Georg-August-University Göttingen, Germany
12
13 **ms. has 28 pages, 2 figures, 5 tables**
14
15 *Correspondening author:
16 Prof. Karl-Heinz Südekum
17 University of Bonn
18 Institute of Animal Science
19 Endenicher Allee 15
20 53115 Bonn, Germany
21 Tel.: +49 228 732287
22 Fax: +49 228 732295
23 ksue@itw.uni-bonn.de

Abstract

In herbivore ecophysiology, comparative chewing efficiency has only recently received increased attention. This measure is best assessed on un-processed forage-only diets; corresponding comparative datasets are missing. We measured faecal mean particle size (MPS [mm]) in 14 large herbivore species (body mass (M) range 60-4000 kg; 8 ruminant and 6 hindgut fermenters) fed a consistent grass hay diet, in which intake, digesta mean retention times (MRT [h]) and digestive efficiency (as digestibility of faecal fibre measured by 96 h cumulative *in vitro* gas production GP96h [ml per 200 mg faecal fibre], and metabolic faecal nitrogen MFN [% organic faecal matter]) had been quantified simultaneously. MPS was generally lower in ruminants than in hindgut fermenters and increased with M in the total dataset, but was nearly constant among closely related taxa (e.g. within ruminants, within equids) irrespective of M. MPS (but not MRT) was significantly correlated to GP96h, whereas MRT (but not MPS) was significantly correlated to MFN, suggesting different effects of these factors on different aspects of digestibility. Combinations of measures including MPS mostly explained digestibility better than other combinations. The phylogenetic signal λ, which was mostly 1 when linking any single measure to digestibility, was estimated 0 in models that linked digestive efficiency to combinations of measures. These results support the intuitive concept that species diversification in large herbivores is tightly related to digestive physiology, and that chewing efficiency as measured by faecal particle size is an integral aspect of this scenario.

Key words: teeth, chewing, herbivory, digestion, foregut fermentation, hindgut fermentation, rumination

Introduction

Large herbivores display a conspicuous diversity within and across ecosystems, with a fascinating variety that includes ruminants, camelids, hippopotamids, suids, equids, rhinocerotids and elephants, to name some prominent groups (Owen-Smith, 1988). Among the attempts to classify this diversity, to explain niche differentiation, but also to understand the substantial convergence between herbivores from different clades, digestive physiology has played a major role. Approaches that build on basic differences in anatomy and physiology have focussed on a dichotomy between hindgut and foregut fermenters (Janis, 1976; Alexander, 1991). More recently, this dichotomy has been expanded by emphasizing differences between nonruminant and ruminant foregut fermenters, and the flexibility of the hindgut fermentation system (Schwarm et al., 2009; Clauss et al., 2010a).

Another approach focuses on variation linked to body mass (M) (Demment and Van Soest, 1985; Illius and Gordon, 1992). In particular, it is assumed that larger herbivores have longer digesta mean retention times (MRT) in the gastrointestinal tract and hence can achieve higher digestive efficiencies. This approach has been criticised because of conceptual problems as well as lacking support from empirical data (reviewed in Clauss et al., 2013; Müller et al., 2013). On the contrary, empirical data suggest no difference in digestive efficiency due to variation in M (Pérez-Barbería et al., 2004; Steuer et al., 2013; Steuer et al., 2014). In particular, it should be noted that the M-concept included the ruminant-hindgut fermenter dichotomy (Illius and Gordon, 1992), and therefore actually allowed for a difference in digestive physiology (or phylogeny).

While MRT was included in these concepts from the very beginning as a crucial physiological factor, chewing efficiency or digesta (= faecal) mean particle size (MPS) was not (Clauss and Hummel, 2005). The relevance of MPS lies in the fact that smaller particles allow a faster microbial digestion due to an increased surface-volume ratio (e.g. Bjorndal et al., 1990), i.e., at a given MRT, smaller MPS should result in higher digestive efficiency. MPS has only been investigated more recently in a comparative approach (Fritz et al., 2009) that demonstrated both a systematic interspecific scaling with M, but also fundamental differences between different herbivore groups. MPS measurements were not included in large-scale comparative studies on the digestive physiology of herbivores (e.g. Foose, 1982), and hence no large-scale comparative study that recorded several digestive measurements including MPS exists so far. For example, conclusions on the compensating effects of MRT and MPS were based on data collated from different studies (Clauss et al., 2009). Here, we report MPS measurements in individuals of a larger number of mammalian herbivores from

experiments during which one consistent (grass hay) diet was fed and food intake and MRT of solute and particle markers (Steuer et al., 2011) as well as proxies for digestibility were recorded simultaneously, the *in vitro* digestibility of faecal fibre (Steuer et al., 2013; a proxy for how thoroughly an animal digested fibre) and metabolic faecal nitrogen (MFN) (Steuer et al., 2014; a proxy for microbial nitrogen which increases with increasing digestibility).

The following hypotheses guided our approach:

1. MPS increases with increasing M (Fritz et al., 2009).
2. MPS is lower in ruminants than in nonruminants (in our sample, nonruminants were all hindgut fermenters) (Fritz et al., 2009).
3. MRT characteristics and MPS together explain digestive efficiency better than M (Clauss et al., 2009). In detail, we expect no influence of M on MRT or digestibility as already shown in these data (Steuer et al., 2011; 2013), a positive relationship between MRT and digestibility (i.e. a negative relationship with *in vitro* digestibility of faeces), a negative relationship between MPS and digestibility (i.e. a positive relationship with *in vitro* digestibility of faeces), and a clear negative relationship between the relative food intake and MRT (Clauss et al., 2007a; 2007b).
4. Given that ruminants and hindgut fermenters in this dataset had a large range of overlap in M, that they differed clearly for *in vitro* digestibility of faecal fibre with no overlap between the groups (Steuer et al., 2013), but that both relative food intake and MRT characteristics, though generally different, did show some overlap (Steuer et al., 2011), we predicted that MPS is a better measure to explain the difference in digestive efficiency between the groups.

In evaluating the effects of the different variables on the digestibility proxies, we used an information-criterion based approach to select the most parsimonious models. Two important aspects were included. First, the models were tested with and without the inclusion of the general digestion type (ruminant vs. hindgut fermenter) as a cofactor. Our premise was that should models be selected that include this information, this would indicate that aspects of these digestive strategies were relevant that are not reflected in the physiological measures included in this study. Second, the models were tested with ordinary statistics and with phylogenetic information using Phylogenetic Generalized Least Squares, which also allowed the estimation of the phylogenetic signal in the investigated models. Our premise was that if a single-factor model contained a significant phylogenetic signal but a model that included several factors in combination did not, this combination of factors likely represents a trait typical for a phylogenetic group. As an evident control example, we expected that in our

dataset, the inclusion of digestion type (which largely reflected the phylogenetic composition of our sample, with elephants and warthogs as individual taxonomic outliers in the hindgut fermenter group) should lead to no significant phylogenetic signal.

Materials and Methods

The experimental setup for this study was described recently (Steuer et al., 2011; 2013; 2014). In brief, 16 species (9 functional ruminants, including 8 taxonomic ruminants and one camelid, and 7 hindgut fermenters) were used on the consistent diet in captivity, with 1-5 individuals per species (Table 1). Sampling periods were during winter seasons 2008 and 2009 at locations in the Netherlands, northern Germany, and Switzerland. Faecal samples were taken after an adaptation period of 14 days during which all animals had *ad libitum* access to a grass hay that was fed whole (i.e., not chopped). Chemical composition (in % organic matter [OM] ± standard deviation) of the grass hay was: neutral-detergent fibre (NDF) 72 ±4, acid-detergent fibre 39 ±4, acid-detergent lignin 5 ±1 and crude protein 10 ±2. Details of this part of the study can be found in Steuer et al. (2011). Body mass of the animals ranged from 58 kg (a domestic goat) up to 6500 kg (an African elephant bull). For ten of the 16 species, M were estimated (from estimations by zoo keepers, zoo veterinarians and the second author, based on literature data and personal experiences); for the rest, individuals were weighed for the experiment. It was logistically not possible to weigh animals before and after the diet transition; based on visual judgements of animal keepers, the supervising veterinarian and the second author, no animal lost body condition during the experiment. For 14 species, data were also available on intake, measured as dry matter intake (DMI [kg d^{-1}]), MRT of a solute (fluid) and a particle marker, and the digestibility of faecal fibre measured by 96 h cumulative *in vitro* gas production (GP96h [ml per 200 mg faecal NDF]), as well as the concentration of metabolic faecal nitrogen (MFN [%faecal OM]) (Steuer et al., 2011; 2013; 2014) (Table 1). Note that a higher GP96h value indicates a higher digestibility of faecal fibre *in vitro*, which in turn means a lower fibre digestibility achieved by the animal; in contrast, a higher MFN reflects a higher organic matter digestibility. Because the ratio between the $MRT_{particle}$ and MRT_{solute} is likely an important digestive measure (Müller et al., 2011), it was expressed as the selectivity factor ($SF = MRT_{particle}/MRT_{solute}$ [no unit]).

Sieve analysis of faecal samples and the calculation of the MPS ([mm]) were performed as described by Fritz et al. (2012) for the discrete mean (dMEAN), with a series of sieves of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.063 mm (linear dimensions of holes), using a Vibrotronic Typ VE 1 (Retsch, Haan, Germany). Sieving time was 10 minutes using an amplitude of

approximately 2 mm and a water throughput of 2 L/min. The size of the largest particles retained on the largest sieve was recorded using a ruler (maximum particle size). Residuals on sieves were transferred onto tared filter paper, dried to constant weight and weighed. In the case of elephants and rhinos, in which soil intake from the enclosure was possible and in whose samples sand was observed on the finer sieves, a correction for sand was performed by ashing the respective sieve samples at 550°C for 5 h (method 8.1; VDLUFA, 2007) and subtracting the remainder from the original weight of the residue on the respective sieve. The results were first expressed as the proportion of particle mass retained on each sieve *p(i)* of the total mass of particles retained on all sieves. Note that, in general, the approaches presented here are based on mass measurements and hence on average particle size per unit mass, not per number of particles. For calculating the dMEAN, sieves are ordered from size *S(*1*)* (minimum) up to size *S(n)* (maximum pore size). The proportion *p(i)* of particles retained at the size *S(i)* includes the particles smaller than *S(i* + 1*)* but not those larger than *S(i)* ('cumulative oversize'). The mean size of these particles is the mean between *S(i* + 1*)* and *S(i)*. The mean size of particles retained at the maximum sieve size *S(n)* is estimated in the same way with the maximum particle size *S(n* + 1*)*. The dMEAN is then calculated by multiplying these mean sizes with the respective proportions and adding up the results:

$$dMEAN = \sum_{i=1}^{n} p(i) * \frac{S(i+1) + S(i)}{2}$$

In a first step, the allometric relationship between MPS (= dMEAN) and M as $MPS = a * M^b$ was assessed using species means for all 16 species by linear regression of log-transformed values using both Ordinary Least Squares (OLS) and Phylogenetic Generalized Least Squares (PGLS) (Martins and Hansen, 1997; Rohlf, 2001). For PGLS analyses, data were linked to a supertree of extant mammals (Bininda-Emonds et al., 2007, 2008), which was pruned to include only the species of concern for our study, using Mesquite (Maddison and Maddison, 2006). The two different domestic horse breeds were represented as direct relatives in the tree. Because the resulting trees were not based on our own calculations of branch lengths with consistently the same characters, we used trees with branch lengths set to 1. We obtained the value of phylogenetic signal (λ) (Pagel, 1999; Revell, 2010) estimated with maximum likelihood (Revell, 2010), using the PGLS command from the package *caper* (Orme et al., 2010). Generally, λ varies between 0 (no phylogenetic signal) and 1 (the observed overall pattern in the dataset is predicted by the phylogeny; similarity among species scales in proportion to their shared evolutionary time) (Pagel, 1999; Freckleton et al., 2002). Due to the low species number, PGLS was not applied within hindgut fermenters or

ruminants only, respectively.

In a second step, the dataset of species means was used in which M, MPS, DMI, MRT (which denotes, from now on, $MRT_{particle}$), SF, GP96h and MFN were available. Due to recent findings that DMI in large herbivores does not scale to $M^{0.75}$, but to a higher exponent between 0.83 and 0.90 (Hackmann and Spain, 2010; Steuer et al., 2011; Müller et al., 2013; Riaz et al., 2014), the relative DMI (rDMI) was calculated on a $M^{0.85}$ basis. This was justified in the dataset, because using species means, this exponent was 0.85 (95% confidence interval 0.71 - 0.99) in OLS and 0.80 (0.67 - 0.92) in PGLS. Body mass and MPS were used after log-transformation.

Initially, correlations between pairs of measures were tested in OLS and PGLS. Then, a set of General Linear Models (GLM) was tested with GP96h or MFN as the dependent variable, and all possible combinations of M, rDMI, MRT, SF, MPS, and digestion type (ruminant or hindgut fermenter) as independent variables/factors. Models were compared for goodness-of-fit using the Akaike's Information Criterion (AIC). Following guidelines published for wildlife research, we selected as best-supported models those with a ΔAIC score of ≤ 2, where ΔAIC = AIC - minimum AIC within the candidate model set (Burnham and Anderson, 2001, 2002). Model comparisons are presented with and without inclusion of models that consider digestion type.

Statistical tests were performed in SPSS 21.0 (SPSS Inc., Chicago, IL) and R 2.15.0 (Team, 2011) using the packages *ape* (Paradis et al., 2004), *caper* (Orme et al., 2010) and *nlme* (Pinheiro et al., 2011). In contrast to a common recommendation (Freckleton, 2009), we display results of both OLS and PGLS analyses, to elucidate which digestive variables are tightly related to phylogeny.

Results

Using means for all 16 species, the resulting allometric exponent for MPS was $M^{0.49}$ in OLS and $M^{0.25}$ in PGLS; however, the PGLS allometry was not significant (Table 2), indicating that there was no allometric relationship among closely related species, such as the ruminants or the equids, and that the effect observed in OLS was due to the distribution of unrelated species across the data space. Correspondingly, there was no significant allometry (in OLS) in ruminants only (Table 2).

In the correlation analyses, there was no relationship between M and any other measure, except for MPS in OLS; in PGLS, again, the relationship was not significant (Table 3). Again, correlation analysis for ruminants only confirmed no significant relationship (Footnote to

Table 3). However, the phylogenetic signal λ was 1.0 or slightly below in all M relationships, indicating that whereas there was no significant correlation with M among closely related species, the overall dataset was structured by separate groups of related species (as evident in Fig. 1).

rDMI was significantly correlated to MRT in both OLS and PGLS; λ was 0.7 (Table 3). This indicates that whereas the overall dataset is structured by separate groups of related species, the relationship is valid across the whole dataset as well as among closely related species. In contrast, the significant negative relationship between rDMI and MFN (relating higher food intake levels with lower digestibility) yielded a λ of 0, indicating that in this case, there was no phylogenetic structure in the dataset. In contrast, significant relationships of rDMI with SF and GP96h in OLS were not found in PGLS (Table 3).

$MRT_{particle}$ correlated positively with SF and with MFN in OLS and PGLS, indicating that longer $MRT_{particle}$ is linked to a more pronounced SF, and to a higher digestibility. Again, there was no relationship with the other digestibility proxy GP96h (Table 3). The SF was negatively related to GP96h and positively to MFN, both in OLS and PGLS; the first relationship had a high phylogenetic signal, and the second had none, indicating a different clustering of closely related species in these datasets.

The MPS-GP96h relationship clearly separated the ruminants from the nonruminants with no data range overlap (Fig. 1); this relationship was significant in both OLS and PGLS (Table 3). In contrast, there was no significant correlation between MPS and MFN; in this case, the two largest hindgut fermenters – the white rhino and the elephant – were clear outliers with higher MFN values than expected based on their large MPS (Fig. 1). The two digestibility proxies GP96h and MFN were negatively related, as would have been predicted, in both OLS and PGLS (Table 3).

The highest-ranking models for the effect on digestibility always included digestion type (Tables 4 and 5). When evaluating effects on GP96h (Table 4), MPS was part of the best models in OLS, regardless of whether digestion type was included or not. MPS was also part of the best PGLS models without digestion type. In the PGLS models that included digestion type, many different models with and without MPS ranked similarly. In all cases, irrespective of whether digestion type was included or not, λ was 0 in the best PGLS models. When evaluating effects on MFN (Table 5), MPS was part of the best OLS models that included digestion type, and part of the best-ranking model without digestion type that included more than one covariable; the best OLS model without digestion type was, however, that only including SF. In the best PGLS models including digestion type, MPS was again included,

whereas the best PGLS models without digestion type included rather SF and rDMI. Again, λ was 0 in the best PGLS models.

Discussion

This study generally confirms the relevance of mean particle size (MPS) in herbivore digestive physiology, while challenging the concept that MPS increases systematically with M among closely related species. It also provides preliminary evidence for the relevance of the degree by which the fluid phase moves faster through the gastrointestinal tract than the particle phase, measured as the selectivity factor (SF, the ratio of particle to solute marker retention) (Steuer et al., 2010), also termed 'digesta washing' (Müller et al., 2011). The measures of mean retention time (closely linked to food intake level), particle size reduction and digesta washing are important characteristics of large mammalian herbivores that reflect, in their influence on digestibility, differences between digestion types without explaining these differences completely. Evidently, additional factors shape the differences between digestion types. Effects of M are not apparent via effects on digesta retention (Müller et al., 2013), but may occur in other ways. For example, whether sheer fermentation volume can have an influence on microbial populations, leading to higher MFN values in the two largest hindgut fermenters of this study in spite of larger MPS (Fig. 1), remains to be investigated. In the different conclusions made here, this study can serve as an example for the concept that in comparative analyses, one should neither consider only results achieved by phylogenetic statistics (as e.g. promoted by Freckleton, 2009), nor discard phylogenetic statistics because biological characters of interest reflect phylogeny (as e.g. suggested by McNab, 2003), but use information from statistical analyses without and with accounting for phylogeny in the interpretation of data patterns.

Due to the close link between the relative dry matter intake (rDMI) and particle mean retention time (MRT) also evident in our study, herbivores face the choice of eating a lot – with shorter MRT and less thorough digestion, or eating less – with longer MRT and more thorough digestion. By modifying the effect of intake on MRT, (spare) gut capacity can play an additional role in this dichotomy (Clauss et al., 2007b). However, the similarity in gut capacity between vertebrate herbivores with a low (reptiles) or high (mammals, birds) rDMI suggests that gut capacity is, in evolutionary terms, at its possible maximum (Clauss et al., 2013). Reasons for constraints on maximum gut capacity remain to be elucidated (Clauss et al., 2003). Via MPS, chewing efficiency is another major modifier of the low/high intake dichotomy; a higher chewing efficiency allows a higher rDMI without compromising

digestive efficiency (Schwarm et al., 2009). With no overlap in the MPS measured between ruminants and nonruminant species means (Fig. 1), previous findings were confirmed that with respect to MPS, ruminants are different from other similar-sized herbivores (Udén and Van Soest, 1982; Fujikura et al., 1989; Campos-Arceiz et al., 2004; Fritz et al., 2009). This high degree of particle size reduction and digestive efficiency in ruminants may be one possible reason for their comparatively high methane production when compared to nonruminants (Franz et al., 2010; Franz et al., 2011b). Similar to previous findings (Clauss et al., 2013; Müller et al., 2013; Steuer et al., 2014), concepts that relate digestive physiology mainly to M are not supported by this study.

This study is constrained by the low number of individuals (in several cases, n=1) investigated per species; additional important influence factors for chewing efficiency, such as the age of individuals (e.g. Venkataraman et al., 2014), could also not be taken into account. A similar constraint of this study is its limited number of species. In this respect, it appears inferior to previous data collections (e.g. Müller et al., 2013), and a broader cover of body masses within ruminants, and an inclusion of smaller hindgut fermenters (such as rodents), would have been desirable. However, to our knowledge, our study represents the largest dataset of herbivores that comprises simultaneous measures of rDMI, MRT, SF, MPS and digestibility on a consistent diet, thus largely excluding dietary factors as covariables influencing the results. Nevertheless, for example, species-specific variation, such as in dental design as described for different ruminant species (e.g. Kaiser et al., 2010), could have potentially overridden small effects of body size on MPS in this study. The use of a consistent diet may not be representative for natural conditions, where even species of the same feeding type (such as ‘grazers’) may select different diet items, the characteristics of which may affect chewing efficiency (e.g. Venkataraman et al., 2014). Ideally, experimental studies like ours should be complemented by comparative data from free-ranging animals choosing their natural diet.

Another limitation of the study design is that digestibility was only estimated from residual fibre digestibility (GP96h) or metabolic faecal nitrogen (MFN), without direct quantification of the digestive efficiency. Such direct quantification would require reliable measuring of faecal output over a period of several consecutive days on an individual basis (Robbins, 1993), with either total faecal collection or the continuous, quantitative application of a digestibility marker – a logistical challenge beyond the possibilities of this study. Using faecal particle size as a proxy for chewing efficiency – without considering the actual time spent chewing required to achieve that particle size –ignores that chewing efficiency is not

only defined by the resulting particle size, but also by the effort required to produce it (e.g. Gross et al., 1995).

Another limitation of the study design is that due to the low number of (closely related) species, it is difficult to tell whether measures contribute to statistical models because they have a real physiological effect, or because they simply act as a proxy for another, unrecognized feature. Therefore, we included digestion type as a cofactor, and suggested that if physiological measures are included in models in addition to this cofactor, they are more likely to have such a real effect. However, a closer look at how MPS contributes to the models in Tables 4 and 5 negates this logic: On the one hand, the effect of MPS in GP96h-models (Table 4) is similar to the simple correlation between these measures – an increase in MPS leading to an increase in GP96h, i.e. a decrease in fibre digestibility achieved by the animals (Fig. 1, Table 3), as would be expected based on physiologic principles (Bjorndal et al., 1990). But on the other hand, the effect of MPS in MFN-models (Table 5) is different; whereas Fig. 1 and Table 3 indicate no relationship, i.e. no systematic change of MFN with MPS (not expected based on physiological principles), MPS is mostly positively related to MFN in the multifactorial models listed in Table 5. This is due to the fact that the two hindgut fermenting species with the largest MPS have higher MFN values than expected based on their MPS. Thus, in the models in Table 5, MPS yields an additional significant discrimination criterion ('very large hindgut fermenter'), that is against common functional logic. Evidently, caution is needed when interpreting the relevance of individual measures in the different explanatory approaches.

A reason for the high explanatory power of 'digestion type' for the fibre digestion efficiency measured is probably that not only MRT, MPS or SF determine this efficiency, but also other additional factors related to digestion type. One important difference concerns the fate of nutrients that the herbivore could digest with its own enzymes, such as plant protein and non-fibre carbohydrates such as starch or sugars. These nutrients are available for the microbes in a foregut but not in a hindgut fermentation system (as they have already been digested and absorbed by the hindgut fermenter in the small intestine). It is known from *in vivo* and *in vitro* experiments that the digestibility of low-quality forages increases when some easily digestible carbohydrates (e.g. Stewart et al., 1979; Miura et al., 1983) or protein (e.g. Silva and Ørskov, 1988; Silva et al., 1989) are supplemented. Theoretically, therefore, fibre digestibility should be higher in a foregut system even if similar retention times and particle sizes as in a hindgut system are assumed. To assess this effect comparatively with a larger

number of species, controlled feeding studies with a forage and graded amounts of a supplement would have to be performed.

Large animals have larger MPS, which corresponds to findings reported and reviewed by Fritz et al. (2009), and animals with a higher rDMI have shorter MRT, which corresponds to both intra- and interspecific findings reviewed by Clauss et al. (2007a); (2007b) and reported in various studies since (Clauss et al., 2010b; Franz et al., 2011a; Hebel et al., 2011; Sawada et al., 2011; Steuer et al., 2011; Clauss et al., 2014b). Other well-documented relationships were not evident in this study on the level of closely related species, leading to non-significant results in PGLS. Among these is the one between MRT and digestibility, evident *in vivo* both intra- and interspecifically (Udén et al., 1982; Pearson et al., 2001; Munn and Dawson, 2006; Kim et al., 2007; Clauss et al., 2008; Clauss et al., 2009; Müller et al., 2013; Clauss et al., 2014b) and also in *in vitro* studies (Hummel et al., 2006), but notably also not always significant in other studies when phylogenetic relationships of the species investigated were taken into account (significant in PGLS in Clauss et al. (2009), but not in the larger and less homogenous dataset of Müller et al. (2013)). These discrepancies are at least partially due to the different proxies used for 'digestibility'. In the present study, MRT was not correlated in PGLS with GP96h – a proxy for fibre digestibility. However, it was related significantly in PGLS to MFN – a proxy for the overall digestibility (Steuer et al., 2014). For MPS, with a well-documented effect on digestibility in *in vitro* studies (Bjorndal et al., 1990; Bowman and Firkins, 1993; Ellis et al., 2005), the situation was the opposite: no correlation in PGLS with MFN, but a significant one with GP96h. These results could tentatively be interpreted as indicating that for fibre digestion (given the range of retention times of large herbivores), MPS is the more crucial factor, whereas for overall digestibility and microbial growth (as reflected in MFN), variation in MRT is more relevant.

The only measure of this study that was significantly related in PGLS to both GP96h and MFN was the SF (Table 3), indicating that a higher degree of 'digesta washing', i.e. a larger difference between particle and fluid retention, is linked to both a higher fibre digestibility and a higher overall digestibility and microbial growth. It has been suggested that a higher degree of digesta washing removes microbes faster from the fermentation chamber and hence selects for a microbial population that grows faster and is metabolically more active and efficient (reviewed in Clauss et al., 2010a; Müller et al., 2011). This matches the observation that high SF are linked to grass intake (Clauss et al., 2006; Clauss et al., 2010b; Steuer et al., 2010), because grass contains comparatively high levels of digestible, yet slowly-digestible, fibre (Hummel et al., 2006). Yet, more experimental work on the relationships between

384 various aspects of digestive physiology and digestibility proxies is required before such
385 hypotheses can be accepted. The differences between comparative analyses such as ours and
386 experimental assays may well stem from the fact that while the physical mechanisms behind
387 the simple relationships (MRT-digestibility; MPS-digestibility; SF-digestibility) are valid,
388 species may vary in more than just these characteristics and hence not necessarily show the
389 underlying simple relationships consistently in a comparative data set. In the wild, other
390 factors related to oral morphology, physcial access to food and the characteristics of the
391 natural diets may also have such an overriding effect.
392 The results for the relationship between MPS and M (Tables 2 and 3) deserve closer
393 scrutiny. It is well recognized that general (allometric) trends in comparative datasets may
394 indicate a steeper relationship at high taxonomic levels (e.g. at the order level) than is found at
395 a lower taxonomic level (e.g. at the family or genus level) among more closely related species
396 (e.g. Fig. 26 in Fortelius, 1985; Fig. 1d and 3b in Clauss et al., 2014a). This corresponds to the
397 result indicated in Table 2, where the allometric exponent in PGLS is distinctively lower (and
398 even non-significant) than the one in OLS. It is also noteworthy that the addition of two
399 species in Table 2 leads to a decrease of the *P*-value in OLS but an increase of the *P*-value in
400 PGLS, as also reported in other studies (Clauss et al., 2014a). The visual impression of the
401 data (Fig. 1) matches the result of the allometric analysis (Table 2) as well as the correlation
402 analyses in the two digestion types (footnote to Table 3). In closely related species, such as
403 the ruminants or the equids, there appears to be little increase of MPS with M. The concept
404 that MPS is less affected by M, and more by morphophysiological characteristics of
405 individual herbivore groups, can be demonstrated more clearly using a larger data set on
406 individual equids, rhinoceroses and elephants recalculated as the discrete mean from Fritz et
407 al. (2009; Fig. 2). The data pattern indicates no M-effect on MPS within the three groups, but
408 a systematic difference between equids and the other two, which would lead to the
409 assumption of a clear positive M-MPS relationship if the relatedness of the individuals
410 measured was not accounted for. Together with the finding that other biological measures,
411 such as MRT in the gut of herbivores (Clauss et al., 2007a; Müller et al., 2013), herbivore
412 chewing cycle duration (Gerstner and Gerstein, 2008), age at first reproduction (Duncan et al.,
413 2007), gestation period (Clauss et al., 2014a), or longevity (Lemaître et al., 2014) have a
414 lower M-scaling than expected when accounting for the phylogenetic structure of the datasets,
415 this finding suggests that species diversification and niche differentiation is probably less
416 driven by differences in M and more by differences in organismal design.

Two sets of statistical results are of particular interest in this study, and have, to our knowledge, not been emphasized explicitly in the scientific literature so far. First, when comparing different models to explain differences between species in OLS (using AIC scores), using the plain dichotomy of ruminant versus hindgut fermenter (with the latter being, in our dataset, akin to 'nonruminant') yielded distinctively better models than combinations of other physiological measures. This suggests, as stated above, that the measures taken in this study do not provide a complete characterisation of digestion type. Additionally, the fact that digestion type models provide the best explanation for the observed patterns of digestibility suggests that herbivore diversification, and hence taxonomic diversity, is strongly linked to digestive physiology, creating a distinct dichotomy between ruminant and nonruminant herbivores. Rumination clearly is not simply a subtle refinement of previously existing digestive strategies, but represents a fundamental innovation that sets ruminants apart from other herbivores (Schwarm et al., 2009; Clauss et al., 2010a; Schwarm et al., 2013).

However, the measures taken in this study nevertheless represent major features of digestion types, as evidenced by the other remarkable statistical observation. This observation concerns the maximum likelihood estimate of the phylogenetic signal λ. In the individual 2-way relationships between measures of digestive physiology (Fig. 1), λ was often of a magnitude of 1 or close to 1 (Table 3), indicating that the data patterns were clearly structured according to the phylogenetic relationships of the species investigated. In the case of significant relationships in OLS but non-significant relationships in PGLS, a λ of 1 indicates that the effect in OLS is due to a data pattern that occurs at higher taxonomic levels (e.g. at order level) but not within lower taxonomic levels (e.g. within family or genus level) of the dataset in question. This is evident in the ruminant-nonruminant dichotomies (Fig. 1). Such a result necessarily implies that the two measures used in the relationship do not, in their combination, mirror the complete taxonomic pattern in the dataset, but that in addition to their relationship, an additional 'phylogenetic signal' is present. If, however, a character is used as a cofactor in PGLS models that fundamentally codes for the phylogenetic structure of the dataset, such as 'digestion type' (ruminant vs. hindgut fermenter, or in other words ruminant vs. nonruminant) in our case, this factor will be significant at a λ of 0 (Tables 4 and 5). Therefore, the fact that 'digestion type' leads to a λ estimate of 0 may not come as a surprise.

In contrast, the finding that a combination of other measures of digestive physiology as independent variables explaining a digestibility proxy yielded a λ estimate of 0 is remarkable. Actually, a similar result was previously reported in a dataset combined from various feeding

regimes for the combination of fibre digestibility, MRT and MPS (Clauss et al., 2009) but was not emphasized. Notably, in that previously analysed dataset, slightly different taxonomic groups were covered (ruminants including cervids but without caprids; artiodactyls including the hippopotamus but without a suid; rhinocerotids and proboscids both represented by two species). A parsimonious interpretation is that the combination of these two measures of digestive physiology, in relation to fibre digestibility, actually mirrors the taxonomic diversification of large herbivores in these datasets; similar observations were made in the dataset of our study here (Table 4 and 5). Thus, the results of this study support the interpretation that species diversification in herbivores occurred along a gradient of digestive physiology that sets the ruminant system apart from that of the hindgut fermenters (and, possibly, other nonruminants). In other words: other differences between ruminants and nonruminants, such as reproductive strategies (Clauss et al., 2014a) or behavioural/anatomical characteristics (Janis, 1982), notwithstanding, the assumption that digestive physiology was a major factor in lineage diversification and the evolution of large herbivores – in particular that of ruminants (Janis, 1976; Illius and Gordon, 1992; Janis et al., 1994; Clauss et al., 2010a) – is strongly supported, and the increased chewing efficiency of the rumination strategy most likely played a decisive role in this process.

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688 **Table 1** Dataset (means ± standard deviations) on measures of digestive physiology in wild and domestic herbivores of different digestion type (DT,
689 ruminants [RUM] or hindgut fermenters [HF]) used in this study, combining original results on body mass (M [kg]) and mean faecal particle size
690 (MPS [mm]) and previously reported results on dry matter intake (DMI [kg d^{-1}]), particle and solute mean retention time (MRT [h]), faecal fibre
691 digestibility (measured as cumulative *in vitro* gas production over 96 h, GP96h [ml per 200 mg organic matter]) and metabolic faecal nitrogen
692 (MFN [% organic matter]) from the same experiment (Steuer et al., 2011; 2013; 2014).

Species	**n**	**DT**	**M**	±SD (range)	**MPS**	±SD	**DMI**	±SD	**MRT_{part}**	±SD	**MRT_{sol}**	±SD	**GP96h**	±SD	**MFN**	±SD
Camelus bactrianus	4	RUM	450*	0	0.54	0.115	-	-	-	-	-	-	-	-	-	-
Bos primigenius taurus	3	RUM	1287	25 (1260-1310)	0.38	0.165	8.02	1.15	75	4.9	35	0.6	8.5	1.85	1.60	0.07
Syncerus caffer nanus	1	RUM	350*	-	0.31	-	5.52	-	51	-	21	-	13.9	-	1.68	-
Kobus ellipsiprymnus	2	RUM	210*	42 (180-240)	0.27	0.044	2.36	0.34	52	13.4	27	10.6	13.3	4.24	1.79	0.23
Connochaetes taurinus	2	RUM	160*	0	0.45	0.028	3.20	0.31	42	2.8	31	9.2	13.9	0.64	1.65	0.02
Oryx gazella	2	RUM	175*	21 (160-190)	0.52	0.223	2.13	0.12	64	2.1	32	5.7	11.1	0.99	1.57	0.07
Hippotragus niger	2	RUM	175*	21 (160-190)	0.43	0.135	1.86	0.20	54	21.2	33	14.8	12.7	2.33	1.69	0.33
Capra aegagrus hircus	3	RUM	60	2 (58-62)	0.34	0.071	1.09	0.16	51	6.2	30	3.8	13.1	2.41	1.16	0.06
Ovis orientalis aries	3	RUM	94	4 (91-99)	0.26	0.027	1.20	0.31	54	4.0	34	2.1	19.3	7.60	1.42	0.24
Loxodonta africana	5	HF	4000*	1458 (3000-6000)	4.98	1.064	49.90	8.17	30	6.0	29	4.3	28.1	1.26	1.33	0.12
Phacochoerus africanus	1	HF	77	-	1.22	-	1.72	-	44	-	34	-	33.1	-	0.99	-
Equus grevyi	4	HF	390*	20 (380-420)	1.55	1.248	8.09	2.61	28	7.3	25	8.7	25.3	1.87	1.11	0.14
Equus ferus przewalskii	3	HF	250*	0	1.20	0.454	-	-	-	-	-	-	-	-	-	-
Equus ferus caballus (horse)	5	HF	571	52 (488-629)	1.12	0.115	9.74	2.517	26	3.9	22	5.4	26.8	3.86	0.90	0.14
Equus ferus caballus (pony)	3	HF	97	6 (90-101)	1.07	0.106	2.24	0.600	26	1.0	20	1.0	24.8	0.55	1.10	0.09
Ceratotherium simum	1	HF	2000*	-	5.10	-	20.03	-	50	-	34	-	27.6	-	1.58	-

693 *estimated body masses

694 **Table 2** Results of allometric regression analyses (incl. *t*-statistic and *P*-value) for the link
695 between mean faecal particle size (MPS [mm]) and body mass (M [kg]) according to
696 $MPS = a * M^b$, using log-transformed variables and linear regression. 95% confidence
697 intervals indicated in brackets. Analyses performed using ordinary least squares (OLS) or
698 phylogenetic generalized least squares (PGLS).

Dataset	Statistic	*a*	*t*	p	*b*	*t*	p
16 species	OLS	0.05 (0.01; 0.34)	-3.0206	0.009	0.49 (0.15; 0.84)	2.7909	0.014
	PGLS	0.50 (0.03; 7.21)	-0.5135	0.616	0.25 (-0.07; 0.57)	1.5023	0.155
RUM (n = 9)	OLS	0.28 (0.08; 0.99)	-1.9733	0.089	0.05 (-0.18; 0.28)	0.4394	0.674
HF (n = 7)	OLS	0.12 (0.02; 0.58)	-2.6173	0.047	0.45 (0.19; 0.71)	3.4217	0.019
14 species*	OLS	0.04 (0.01; 0.33)	-3.0260	0.011	0.49 (0.14; 0.84)	2.7720	0.017
	PGLS	0.46 (0.05; 4.58)	-0.6615	0.521	0.26 (-0.01; 0.54)	1.8605	0.087
RUM (n = 8)	OLS	0.32 (0.11; 0.99)	-1.9771	0.095	0.02 (-0.19; 0.22)	0.1473	0.888
HF (n = 6)	OLS	0.15 (0.03; 0.68)	-2.4430	0.071	0.41 (0.17; 0.66)	3.3325	0.029

699 *without *C. bactrianus* and *E. przewalskii* (for which additional data for further analyses were
700 not available)
701 RUM = ruminant, HF = hindgut fermenter
702

Table 3 Correlations between various measures of digestive physiology in this study, using species means (n = 14) and Ordinary Least Squares (OLS) or Phylogenetic Generalized Least Squares (PGLS). Body mass (M [kg]), relative dry matter intake (rDMI, [g $kg^{-0.85}$ d^{-1}]), particle mean retention time (MRT [h]), the selectivity factor (SF [no unit]), faecal mean particle size (MPS, [mm]), the digestibility of faecal fibre measured as cumulative gas production during 96 hour *in vitro* fermentation (GP96h, [ml per 200 mg organic matter]), and metabolic faecal nitrogen (MFN [% organic matter]). Significant correlations set in bold; differences between OLS and PGLS indicated by grey shading.

		rDMI	MRT	SF	LogMPS	GP96h	MFN
LogM	OLS	r = 0.006	r = -0.055	r = -0.101	**r = 0.625**	r = 0.203	r = 0.175
		p = 0.985	p = 0.852	p = 0.731	**p = 0.017†**	p = 0.487	p = 0.550
	PGLS	r = 0.142	r = -0.155	r = 0.285	r = 0.399	r = 0.145	r = 0.381
		p = 0.317	p = 0.303	p = 0.979	p = 0.065	p = 0.501	p = 0.077
		λ = 0.741	λ = 1.000*	λ = 1.000*	λ = 1.000*	λ = 1.000*	λ = 0.931*
rDMI	OLS		**r = -0.895**	**r = -0.669**	r = 0.496	**r = 0.678**	**r = -0.664**
			p < 0.001	**p = 0.009**	p = 0.071	**p = 0.008**	**p = 0.010**
	PGLS		**r = -0.835**	r = 0.372	r = 0.285	r = 0.274	**r = -0.655**
			p < 0.001	p = 0.083	p = 0.979	p = 0.170	**p = 0.002**
			λ = 0.784	λ = 0.902	λ = 1.000*	λ = 1.000	**λ = 0****
MRT	OLS			**r = 0.793**	**r = -0.534**	**r = -0.718**	**r = 0.655**
				p = 0.001	**p = 0.049**	**p = 0.004**	**p = 0.011**
	PGLS			**r = 0.798**	r = -0.288	r = 0.333	**r = 0.593**
				p < 0.001	p = 0.997	p = 0.114	**p = 0.006**
				λ = 0	λ = 1.000*	λ = 1.000	**λ = 0****
SF	OLS				**r = -0.666**	**r = -0.776**	**r = 0.665**
					p = 0.009	**p = 0.001**	**p = 0.009**
	PGLS				r = -0.260	**r = -0.626**	**r = 0.606**
					p = 0.186	**p = 0.004**	**p = 0.005**
					λ = 1.000*	**λ = 0.806**	**λ = 0**
LogMPS	OLS					**r = 0.788**	r = -0.359
						p = 0.001	p = 0.207
	PGLS					**r = 0.772**	r = 0.288
						p < 0.001	p = 0.994
						λ = 0	λ = 1.000
GP96h	OLS						**r = -0.682**
							p = 0.007
	PGLS						**r = -0.618**
							p = 0.004
							λ = 0

*λ significantly different from 0; **λ significantly different from 1 ($p < 0.05$)

†correlation analyses in only ruminants and only hindgut fermenters in OLS yielded no significance in ruminants ($r = 0.060$, $p = 0.888$, n = 8) and significance in hindgut fermenters ($r = 0.857$, $p = 0.029$, n = 6).

Table 4 Selected general linear models (incl. 95% confidence intervals [CI]) (n = 14 species), with the digestibility of faecal fibre measured as cumulative gas production during 96 hour *in vitro* fermentation (GP96h [ml per 200 mg organic matter]) as the dependent variable and various combinations of (log-transformed) body mass (M [kg]), relative dry matter intake (rDMI, [g $kg^{-0.85}$ d^{-1}]), particle mean retention time (MRT [h]), the selectivity factor (SF [no unit]) and (log-transformed) faecal mean particle size (MPS, [mm]) as covariables, and digestion type (DT, ruminant coded as 1 or hindgut fermenter coded as 2) as cofactor, in Ordinary Least Squares (OLS) or Phylogenetic Generalized Least Squares (PGLS), ranked according to their Akaike's Information Criterion (AIC), compared with and without DT models.

Model					Δ AIC		a			b			c			d		
	a	b	c	d				95%CI			95%CI			95%CI			95%CI	
OLS	DT	M	MPS	SF	0.00		12.12	4.31	19.93	-2.89	-7.75	1.98	3.40	-7.62	14.41	-1.49	-8.37	5.39
OLS	DT	M	MPS		2.49		12.66	5.59	19.74	-3.31	-7.57	0.95	4.12	-5.93	14.18			
OLS	DT	MPS	SF		3.00		13.38	5.74	21.03	-1.24	-9.14	6.66	-3.14	-9.54	3.26			
OLS	DT				8.79		14.40	11.19	17.61									
OLS	M	MPS	SF		9.62	0.00	-4.94	-11.26	1.38	15.27	4.56	25.98	-4.94	-13.74	3.85			
OLS	M	rDMI	MPS	SF	12.65	3.03	-4.25	-10.81	2.31	0.14	-0.17	0.46	14.08	2.95	25.21	-3.32	-12.89	6.26
OLS	M	MPS			13.67	4.05	-6.86	-12.23	-1.50	19.94	13.11	26.76						
OLS	MRT	MPS			21.01	11.39	-0.23	-0.42	-0.03	10.41	3.92	16.89						
PGLS*	DT	M	MPS		0.00		11.72	4.70	18.74	-3.82	-7.95	0.31	6.14	-3.36	15.65			
PGLS*	DT				0.05		14.86	11.77	17.95									
PGLS*	DT	M			0.08		15.76	12.46	19.06	-1.71	-4.31	0.89						
PGLS*	DT	SF			1.39		13.25	7.88	18.62	-2.38	-8.79	4.03						
PGLS*	DT	M	SF		1.53		14.33	8.77	19.89	-1.63	-4.32	1.05	-2.05	-8.36	4.26			
PGLS*	DT	M	MPS	SF	1.86		11.32	3.51	19.13	-3.62	-8.13	0.89	5.68	-4.74	16.09	-1.02	-7.57	5.53
PGLS*	DT	rDMI			1.96		14.43	9.84	19.02	0.03	-0.21	0.27						
PGLS*	DT	MPS			1.97		15.53	9.28	21.78	-0.80	-7.20	5.60						
PGLS*	DT	M	MRT	MPS	1.99		11.88	3.05	20.70	-3.81	-8.17	0.54	0.01	-0.16	0.17	6.09	-4.04	16.23
PGLS*	DT	M	rDMI	MPS	2.00		11.72	2.92	20.52	-3.82	-8.20	0.56	0.00	-0.25	0.25	6.14	-3.97	16.26
PGLS*	M	rDMI	MPS		7.89	0.00	-6.32	-11.30	-1.35	0.18	-0.08	0.44	17.10	9.70	24.50			
PGLS*	M	MRT	MPS		8.01	0.11	-6.73	-11.50	-1.97	-0.12	-0.30	0.06	17.26	9.92	24.61			
PGLS*	M	MPS			8.18	0.29	-7.86	-12.45	-3.28	20.25	14.28	26.22						
PGLS*	M	MPS	SF		8.82	0.93	-6.49	-11.79	-1.18	16.35	6.73	25.98	-4.16	-12.24	3.91			
PGLS*	M	rDMI	MPS	SF	9.56	1.66	-5.89	-11.38	-0.40	0.14	-0.16	0.45	15.65	5.84	25.46	-2.19	-11.35	6.97
PGLS*	M	rDMI	MRT	MPS	9.74	1.85	-6.39	-11.62	-1.16	0.11	-0.40	0.62	-0.06	-0.40	0.29	16.92	9.08	24.76
PGLS*	MRT	MPS			13.98	6.09	-0.21	-0.42	0.00	9.45	3.32	15.57						

*λ = 0 in all displayed PGLS cases

Table 5 Selected general linear models (incl. 95% confidence intervals [CI]) (n = 14 species), with the concentration of metabolic faecal nitrogen (MFN [% faecal organic matter]) as the dependent variable and various combinations of (log-transformed) body mass (M [kg]), relative dry matter intake (rDMI, [g $kg^{-0.85}$ d^{-1}]), particle mean retention time (MRT [h]), the selectivity factor (SF [no unit]) and (log-transformed) faecal mean particle size (MPS, [mm]) as covariables, and digestion type (DT, ruminant coded as 1 or hindgut fermenter coded as 2) as cofactor, in Ordinary Least Squares (OLS) or Phylogenetic Generalized Least Squares (PGLS), ranked according to their Akaike's Information Criterion (AIC), compared with and without DT models.

Model					Δ AIC		a			b			c			d		
	a	b	c	d				95%CI			95%CI			95%CI			95%CI	
OLS	DT	MPS			0.00		-0.85	-1.23	-0.48	0.63	0.19	1.07						
OLS	DT	MPS	SF		1.61		-0.71	-1.11	-0.30	0.64	0.22	1.06	0.25	-0.09	0.59			
OLS	DT	M			2.31		-0.49	-0.70	-0.29	0.26	0.07	0.45						
OLS	DT	M	MPS		3.06		-0.75	-1.16	-0.33	0.14	-0.11	0.39	0.41	-0.18	1.00			
OLS	DT				3.31		-0.39	-0.63	-0.16									
OLS	SF				3.58	0.00	0.47	0.17	0.77									
OLS	MPS	SF			6.71	3.13	0.10	-0.30	0.50	0.54	0.13	0.96						
OLS	MRT	MPS			14.59	11.01	0.01	0.00	0.02	-0.01	-0.37	0.35						
PGLS*	DT	MPS	SF		0.00		-0.68	-1.05	-0.32	0.63	0.31	0.94	0.29	-0.03	0.62			
PGLS*	DT	MRT	MPS	SF	1.74		-0.72	-1.13	-0.31	0.00	-0.01	0.01	0.65	0.30	1.00	0.34	-0.07	0.76
PGLS*	DT	MPS			1.83		-0.86	-1.19	-0.52	0.59	0.25	0.94						
PGLS*	DT	rDMI	MPS	SF	1.85		-0.65	-1.09	-0.21	0.00	-0.02	0.01	0.60	0.24	0.97	0.27	-0.09	0.64
PGLS*	DT	M	MPS	SF	1.86		-0.66	-1.08	-0.23	0.04	-0.21	0.28	0.56	-0.01	1.12	0.28	-0.08	0.63
PGLS*	M	rDMI	SF		7.43	0.00	0.15	-0.02	0.33	-0.01	-0.03	0.00	0.32	-0.05	0.68			
PGLS*	M	SF			7.86	0.43	0.18	0.00	0.35	0.50	0.23	0.77						
PGLS*	rDMI				8.62	1.19	-0.02	-0.03	-0.01									
PGLS*	M	MRT	SF		8.95	1.52	0.17	-0.01	0.35	0.01	-0.01	0.02	0.35	-0.11	0.81			
PGLS*	M	rDMI			8.97	1.54	0.11	-0.07	0.28	-0.02	-0.03	-0.01						
PGLS*	rDMI	MPS	SF		9.06	1.63	-0.01	-0.03	0.00	0.21	-0.11	0.54	0.40	-0.07	0.87			
PGLS*	rDMI	SF			9.19	1.76	-0.01	-0.03	0.00	0.21	-0.17	0.59						
PGLS*	M	rDMI	MRT	SF	9.23	1.80	0.15	-0.03	0.33	-0.01	-0.04	0.01	0.00	-0.03	0.02	0.36	-0.09	0.82
PGLS*	M	rDMI	MPS	SF	9.43	2.00	0.16	-0.14	0.45	-0.01	-0.03	0.01	-0.01	-0.54	0.52	0.31	-0.18	0.80
PGLS*	SF				10.06	2.63	0.42	0.14	0.71									

*$\lambda = 0$ in all displayed PGLS cases

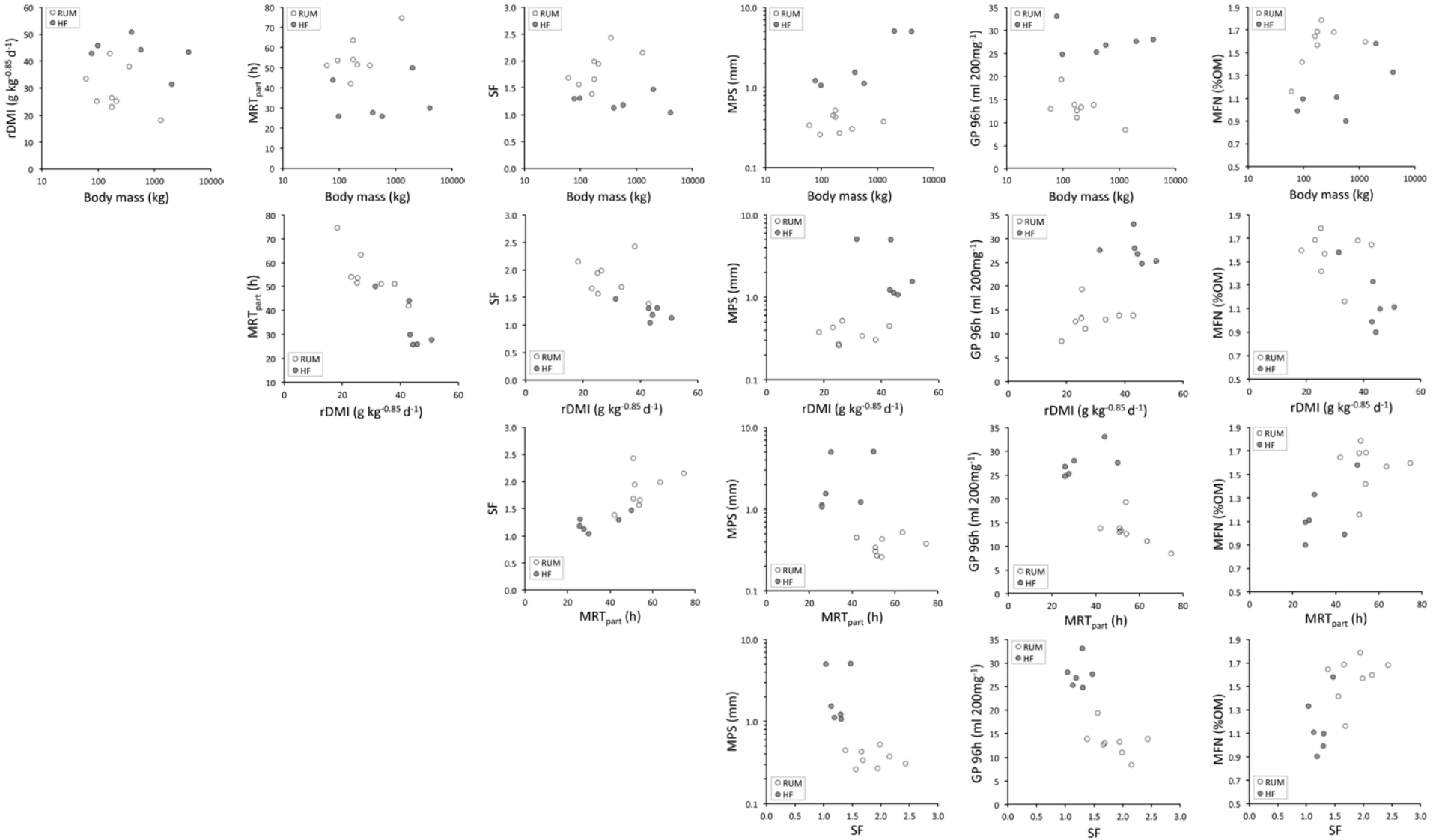
729 **Figure legends**

730

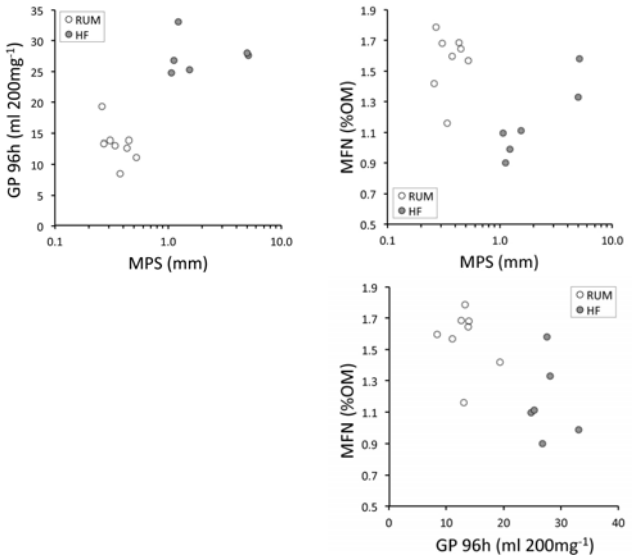
731 **Figure 1 Relationships between various measures of digestive physiology in this study**,
732 using species means (n = 14). Body mass [kg], relative dry matter intake (rDMI [g $kg^{-0.85}$ d^{-1}
733]), particle mean retention time (MRT [h]), the selectivity factor (SF [no unit]), faecal mean
734 particle size (MPS, [mm]), the digestibility of faecal fibre measured as cumulative gas
735 production during 96 hour *in vitro* fermentation (GP96h, [ml per 200 mg organic matter]), and
736 metabolic faecal nitrogen (MFN [% organic matter]). Statistics in Table 3. Note the general
737 grouping of data according to digestion type (ruminants open circles, hindgut fermenters grey
738 circles).

739

740 **Figure 2 Relationship between body mass (M [kg]) and the mean faecal particle size**
741 **(MPS [mm]) in equids (wild equids as well as domestic horses and donkeys, incl. *Equus***
742 ***africanus* spp., *E. grevyi*, *E. hemionus* spp., *E. przewalskii*, *E. quagga* spp., *E. zebra*),**
743 **rhinoceroses (*Diceros bicornis*, *Ceratotherium simum*, *Rhinoceros unicornis*) and**
744 **elephants (*Elephas maximus*, *Loxodonta africana*)** kept at various institutions on various
745 diets based on individual measurements from the study of Fritz et al. (2009). Note that the
746 overall relationship $MPS = a * M^b$ [with 95% confidence intervals] is highly significant (R^2
747 = 0.78, a = 0.03 [0.01, 0.05; p<0.001], b = 0.68 [0.59, 0.78; p<0.001], black regression line),
748 whereas this is not the case in the individual groups (indicated by dashed lines; equids: R^2 =
749 0.17, a = 0.57 [0.24, 1.33; p=0.188], b = 0.11 [-0.03, 0.26; p=0.130]; rhinos R^2 = 0.25, a =
750 42.85 [2.15, 857.04; p=0.016], b = -0.25 [-0.66, 0.16; p=0.225]; elephants R^2 = 0.10, a = 8.95
751 [0.98, 81.85; p=0.052], b = -0.07 [-0.35, 0.21; p=0.630]).


RUM
HF
rDMI (g kg$^{-0.85}$ d^{-1})
MRT$_{part}$ (h)
SF
MPS (mm)
GP 96h (ml 200mg^{-1})
MFN (%OM)
Body mass (kg)

752
753



754
755 **Figure 1 Relationships between various measures of digestive physiology in this study**, using species means (n = 14). Body mass [kg], relative
756 dry matter intake (rDMI [g $kg^{-0.85}$ d^{-1}]), particle mean retention time (MRT [h]), the selectivity factor (SF [no unit]), faecal mean particle size (MPS,
757 [mm]), the digestibility of faecal fibre measured as cumulative gas production during 96 hour *in vitro* fermentation (GP96h, [ml per 200 mg organic
758 matter]), and metabolic faecal nitrogen (MFN [% organic matter]). Statistics in Table 3. Note the general grouping of data according to digestion
759 type (ruminants open circles, hindgut fermenters grey circles).

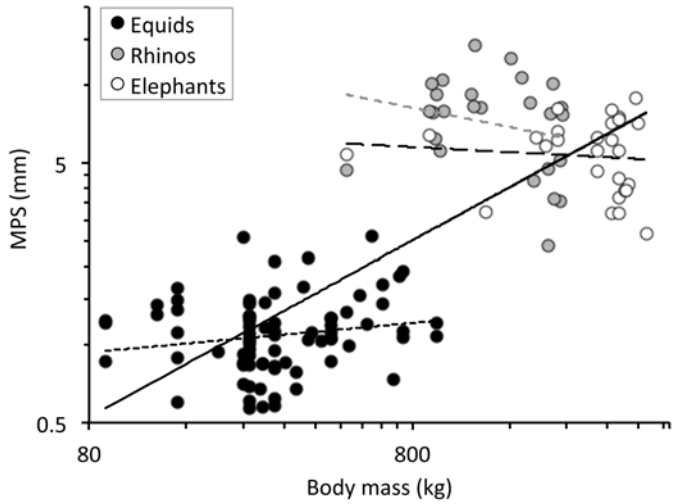


Fig 2 Relationship between body mass (M [kg]) and the mean faecal particle size (MPS [mm]) in equids (wild equids as well as domestic horses and donkeys, incl. *Equus africanus* spp., *E. grevyi*, *E. hemionus* spp., *E. przewalskii*, *E. quagga* spp., *E. zebra*), rhinoceroses (*Diceros bicornis*, *Ceratotherium simum*, *Rhinoceros unicornis*) and elephants (*Elephas maximus*, *Loxodonta africana*) kept at various institutions on various diets based on individual measurements from the study of Fritz et al. (2009). Note that the overall relationship $MPS = a * M^b$ [with 95% confidence intervals] is highly significant (R^2 = 0.78, a = 0.03 [0.01, 0.05; $P < 0.001$], b = 0.68 [0.59, 0.78; $P < 0.001$], black regression line), whereas this is not the case in the individual groups (indicated by dashed lines; equids: R^2 = 0.17, a = 0.57 [0.24, 1.33; P = 0.188], b = 0.11 [-0.03, 0.26; P = 0.130]; rhinos R^2 = 0.25, a = 42.85 [2.15, 857.04; P = 0.016], b = -0.25 [-0.66, 0.16; P = 0.225]; elephants R^2 = 0.10, a = 8.95 [0.98, 81.85; P = 0.052], b = -0.07 [-0.35, 0.21; P = 0.630]).